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^{13}C Nuclear Magnetic Resonance Study of Trehalose Mobilization in Yeast Spores

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Using high-resolution ^{13}C nuclear magnetic resonance, we examined the mobilization of endogenous trehalose in suspensions of yeast asci. Sporulation of yeast cells in [1-^{13}C]acetate resulted in incorporation of label into the C-3 and C-4 positions of trehalose within the asci. During germination of these asci with [1-^{13}C]glucose, the consumption of both endogenous trehalose and exogenous glucose were followed simultaneously by ^{13}C nuclear magnetic resonance, as was the formation of glycerol and ethanol, their glycolytic end products. Time courses for carbohydrate consumption indicated that trehalose, although it decreased to 25% of its initial value upon germination, was not preferentially catabolized and did not provide the primary energy supply for germination with glucose. The ratio of trehalose to glucose catabolized was 0.09. Exogenous glucose levels appeared to regulate trehalose mobilization since trehalose was only consumed when sufficiently high levels (>2 mM) of glucose were present. Upon glucose depletion newly synthesized [1-^{13}C]trehalose was observed. Nuclear magnetic resonance spectra of extracts confirmed the trehalose peak assignments and showed products of [1-^{13}C]glucose catabolism. In addition by quantitating trehalose consumption and 2-deoxyglucose incorporation in dormant yeast asci, we found that 3.8 ± 0.4 molecules of 2-deoxyglucose were incorporated for each trehalose molecule consumed. Trehalose can therefore function as a carbohydrate source for ATP formation during dormancy.

The disaccharide α,α-trehalose accumulates in spores of fungi in remarkably high concentrations (9, 11, 18). During yeast sporulation large quantities of trehalose are synthesized during acetate respiration in a nitrogen-limited medium. There are striking similarities to bacterial spores which contain high concentrations of 3-phosphoglycerate and dipicolinic acid (13). To generalize, it appears that in metabolically dormant spores large amounts of potential substrates for glycolysis are present as well as the full complement of glycolytic enzymes. In both bacteria and fungi these endogenous carbon sources decrease in concentration rapidly upon germination. Bacterial spores quickly catabolize 3-phosphoglycerate and release dipicolinic acid to the medium after heat shock and ionic stimulation (20). Spores of *Neurospora* sp. rapidly glycolyze trehalose after a burst of oxygen (22). Yeast spores require a carbon source for germination (14), and indeed when supplied with an exogenous carbon source, such as glucose, the endogenous trehalose is rapidly depleted (19). Trehalose accumulation also occurs in yeast during periods of diauxie, and upon transfer to an environment facilitating growth the trehalose is degraded (15, 24).

Two possibilities exist for the function of these high concentrations of trehalose. The present experiments have been undertaken in an attempt to distinguish between these two possibilities. The first suggestion, reflecting its decrease during germination, has been that the carbohydrate serves as a rapid endogenous energy supply for the initial stages of germination, or more generally, to begin the growth cycle (11, 15). A second possibility is that trehalose may be important during the dormant period. The ability of trehalose to provide a source of ATP during dormancy may contribute to spore viability. A decrease in trehalose levels in spores of *Neurospora* sp. upon aging has been reported (11). From this result it was suggested that the trehalose was serving as an energy supply during dormancy. Similarly in *Dictyostelium discoideum* spores, it has been shown (6) that trehalose increases the wet heat resistance.

It has been reported (10) that the amounts of glycogen and trehalose in *Saccharomyces cerevisiae* cells are degraded steadily during peri-

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ods of prolonged starvation. The temporal correlation between reserve carbohydrate depletion and the onset of cell death suggested that these carbohydrates may be important in these cells during periods of starvation.

High levels of natural abundant [^{13}C]trehalose have been measured in encysted cells of *Acanthamoeba castellanii* by using high-resolution ^{13}C nuclear magnetic resonance (NMR) (5). We have examined the metabolism of trehalose in asci of the yeast *Pichia pastoris* by using ^{13}C NMR. Utilizing [1-^{13}C]acetate in the sporulation medium, we have obtained a label in the trehalose. The intense ^{13}C NMR signals from the labeled trehalose have been monitored through the germination of the yeast spore with a time resolution of 3 min. The advantages of using the NMR technique are that it is noninvasive (particularly valuable in studying spores [1]), and specific metabolic changes can be monitored on a rapid time scale. In contrast, with radiolabeling (2) or colorimetric (19) assays trehalose levels have been followed during germination by acid extraction, but in these experiments the resolution time was almost 1 h and the subtleties in the temporal relationships between exogenous glucose and endogenous trehalose were not revealed. In this study we used the rapid, quantitative, and noninvasive NMR measurements to investigate trehalose catabolism and elements of its control in yeast spores.

MATERIALS AND METHODS

[1-^{13}C]sodium acetate and [1-^{13}C]glucose, both 90% labeled, were purchased from Merck Sharpe & Dohme and were used without further purification.

Asci of the yeast *Pichia pastoris* strain Y55 were prepared as described previously (1). Asci containing [^{13}C]trehalose were prepared in a similar fashion, but with a sporulation medium containing 1.6% [1-^{13}C]sodium acetate. To increase the yield of ascospores when using sodium acetate the medium was supplemented with 1% potassium phosphate (pH 7.0). In this medium all preparations yielded 94% ascospores.

^{13}C NMR spectra at 90.55 MHz were obtained at 25°C with a Bruker WH-360 NMR spectrometer operating at 90.55 MHz in the FT mode. Pulse intervals used were either 0.5 or 2.0 s, as noted, with a flip angle of 60°. Proton broad band decoupling was employed throughout.

Samples in which germination was followed by NMR consisted of 2.5 ml of either 20% (vol/vol) pellet (high density) or 4% (vol/vol) pellet (low density) ascus suspensions in 50 mM Tris–5 mM potassium phosphate–0.2% antifoam (pH 7.4) in 10-mm outer diameter NMR tubes to which [1-^{13}C]glucose was added. Throughout the experiment, a 95% N_2–5% CO_2 gas mixture was bubbled through the suspension with a fine-tip glass bubbler located at the bottom of the NMR tube below the coil. ^{13}C NMR spectra of the asci suspensions were obtained in 3-min (high density) or 10-min (low density) accumulation times and were continuously stored on a magnetic disk.

Extracts were prepared of asci in the following way: to 7.0 ml of high-density suspension of labeled asci in 50 mM Tris–5mM potassium phosphate (pH 7.4)–0.2% antifoam–[1-^{13}C]glucose was added to a final concentration of 65 mM. The sample was incubated at 25°C with bubbling of the N_2–CO_2 gas mixture and shaking. After 15 min, the glycolyzing asci were quickly added to an equal volume of cold 10% perchloric acid. The suspension was first frozen and then thawed and sonicated for 30 s. This procedure was repeated four times and throughout the sample was kept cold. Thereafter the sample was neutralized with potassium bicarbonate, precipitating the potassium perchlorate salt. The cold suspension was centrifuged in a desk-top centrifuge IEC/HNS II at full speed for 10 min, and the supernatant extract was collected. The ascospore pellet was further extracted by washing three times with cold water and centrifuged, and the extracts were combined. The solution was lyophilized and resuspended in 3.0 ml of 10% D_2O–50 mM EDTA. ^{13}C NMR spectra of the extracts were accumulated by using a 2.0-s pulse interval.

Estimates of the stoichiometry between 2-deoxyglucose incorporation and trehalose usage were made as follows. Freshly prepared, ^{13}C-labeled asci were suspended in 50 mM Tris–5 mM potassium phosphate (pH 7.4)–0.2% antifoam at a density of 20%. Trehalose consumption was measured in an NMR experiment in which NMR spectra were taken during incubation with 16.7 mM 2-deoxyglucose. Spectra were accumulated with pulse intervals of 2.0 s for 10-min periods for up to 9 h, until a constant trehalose level was reached. The fractional reduction in trehalose was calculated from the ratio of ^{13}C-3– and ^{13}C-4–trehalose peak intensities after and before incubation with 2-deoxyglucose. Absolute trehalose concentrations were determined by comparison with the intensity of [1-^{13}C]glucose peaks in spectra taken of samples from the original suspension which were incubated with [1-^{13}C]glucose of known concentration. Trehalose concentrations within the asci determined in this manner compared closely to measurements made using a colorimetric assay of trehalose extracts (8). 2-Deoxyglucose incorporation was determined in a parallel experiment, where the ascus suspension was incubated with 16.7 mM [1,2-^{3}H]2-deoxyglucose. At various time intervals, samples were removed from the incubation mixture and centrifuged at 4°C for 10 min, and the supernatant fraction was counted in Aquasol with a Beckman LS/230 liquid scintillation counter. To determine concentrations, standard solutions of 2-deoxyglucose were also counted. [1,2-^{3}H]2-deoxyglucose was purchased from New England Nuclear Corp. and diluted to a specific activity of 0.02 mCi/mmol with unlabeled 2-deoxyglucose for these experiments.

RESULTS

Both endogenous trehalose and exogenous glucose were followed in the spore simultaneously over the course of germination. Figure 1 shows the ^{13}C NMR spectra of asci containing [^{13}C]trehalose before and after the addition of [1-^{13}C]glucose. The simplicity of the labeling pattern introduced by the acetate label is apparent in Fig. 1a. Here, before germination, only

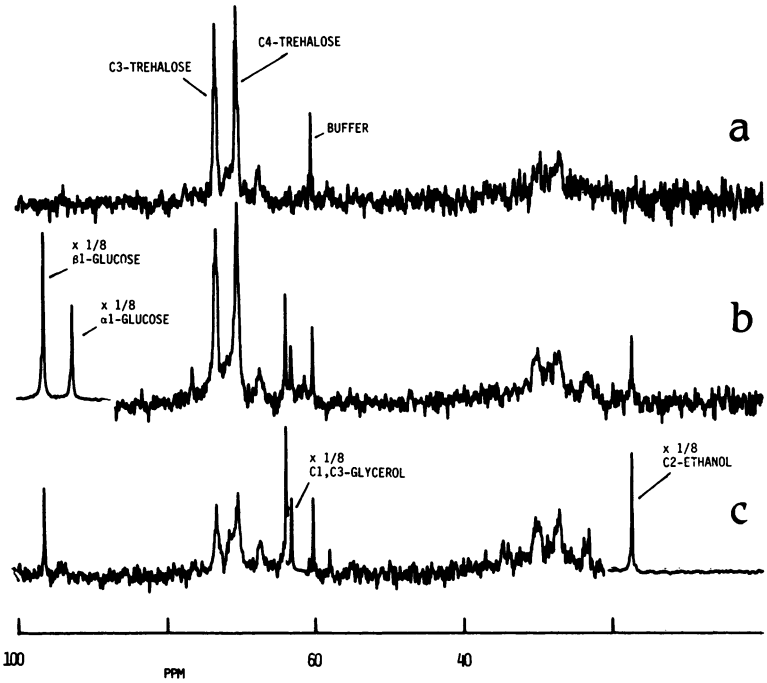

FIG. 1. ^{13}C NMR spectra of a 20% suspension of labeled asci before and during germination with 75 mM [1-^{13}C]glucose in 50 mM Tris–5 mM potassium phosphate (pH 7.4) at 25°C: (a) before germination; (b) accumulations between 4 and 7 min after [1-^{13}C]glucose addition; (c) accumulations between 58 and 61 min (pulse interval, 0.5 s; line broadening, 5 Hz).

the C-3 and C-4 peaks of trehalose were appreciably labeled. Smaller pairs of broad resonances at 71.4 and 67.2 ppm and at 30.1 and 27.4 ppm were also observed, but did not appear in spectra taken of extracts and did not change appreciably over the course of germination. Given their chemical shifts and the likelihood that they are polymeric, the former pair may correspond to glycogen, and the latter pair may correspond to fatty acids, but neither pair is assigned definitively.

The spectrum of labeled asci is expanded in Fig. 2 to show the region of the C-3 and C-4 peaks, which were the only trehalose sites appreciably labeled with [1-^{13}C]acetate as previously observed (3). The full line widths at half-height of the individual peaks centered at 70.6 and 73.4 ppm were only 8 Hz, giving a well-resolved spectrum from both carbons consisting of a single peak from each labeled carbon next to an unlabeled neighbor, plus a doublet which has been split by a labeled neighbor. The measured doublet splitting was 38 Hz. A similar set of three lines was seen at both the C-3 and C-4 positions of trehalose. The existence at one carbon, e.g., C-3, of peaks corresponding to the fractions of its neighbors which were both labeled and unlabeled, plus the measurements of the amount of label at the neighboring C-4 position, enables one to calculate both the labeled and total amounts of trehalose formed from acetate. The peak intensity distribution within each triplet showed a ratio of 5.5:4 for the doublet to the singlet areas, which corresponds to a labeling distribution of 35, 48, and 17% for the doubly labeled C3–C-4, singly labeled C-3–C-4, and unlabeled C-3–C-4, respectively. At these trehalose positions 59% of the carbon atoms were ^{13}C enriched as compared with 90% ^{13}C enrichment in the original acetate label. This dilution of the label corresponds quite closely to the enrichment observed in aerobically metabolizing yeast cells fed [1-^{13}C]acetate, which is not surprising since the dormant ascus represents an end stage of aerobic sporulation. Dilution of the acetate label may be explained by approximately equal fluxes through the tricarboxylic acid and the glyoxylate cycles (3).

Figure 1 shows how ^{13}C NMR may be used to follow separately the consumption of glucose and trehalose during germination. At time zero [1-^{13}C]glucose was added to a concentration of 74 mM and ^{13}C NMR spectra were accumulated for periods of three minutes. Figure 1b shows

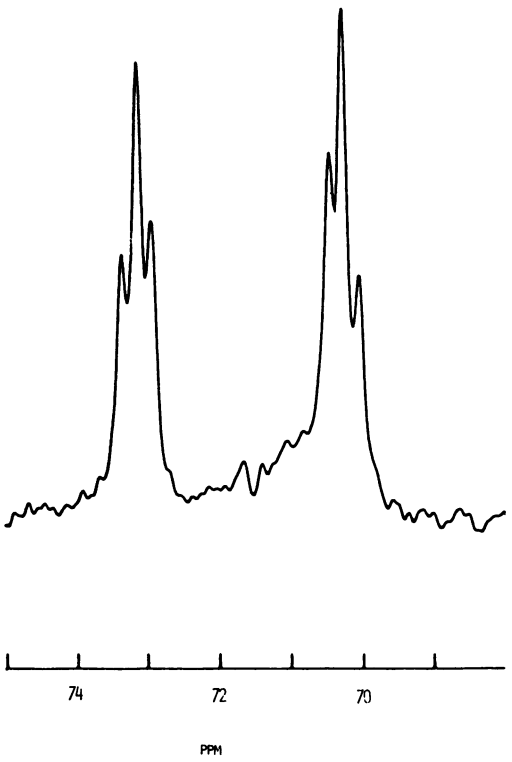

FIG. 2. Expanded region of the proton-decoupled 90.55-MHz ^{13}C NMR spectrum of the labeled ascus suspension. Asci were labeled through sporulation in 1.6% [1-^{13}C]sodium acetate. The resultant label in α,α-trehalose was found in the C-3 and C-4 positions. The spectrum was accumulated for 3 min. Pulse intervals, 0.5 s; line broadening, 5 Hz.

the spectrum accumulated between 4 and 7 min after glucose addition. The [1-^{13}C]glucose peaks from the α and β anomers were very intense and are reduced by a factor of 8 so as to fit on scale. In Fig. 1b one can see the appearance of glycerol and ethanol peaks, the glycolytic end products. Figure 1c shows the spectrum taken between 58 and 61 min after glucose addition when most of the glucose had been consumed and the trehalose reduced appreciably. In this spectrum, peaks from the glycerol and ethanol end products have been reduced eightfold. We emphasize that these NMR spectra clearly distinguish between the levels of glucose, as followed by its 1-^{13}C label, and of trehalose, as followed by its 3-^{13}C and 4-^{13}C peaks. Distinctly labeled products of endogenous trehalose and exogenous glucose consumption cannot be discerned, however. The terminally labeled glycerol carbon atoms are unresolved, and hence the same glycerol peak is derived from both glucose and trehalose degradation. Since the carboxyl carbon atom of pyruvate, derived from either C-3 or C-4 of trehalose, is subsequently eliminated as carbon dioxide through the action of pyruvate decarboxylase, the trehalose label is lost and hence any [2-^{13}C]ethanol observed is derived solely from the [1-^{13}C]glucose. Intermediates of aerobic glycolysis such as glucose 6-phosphate are especially low in concentration during germination, and therefore with the sensitivity available distinct intermediates from either trehalose or glucose usage could not be distinguished. It is clear, however, that trehalose is catabolized glycolytically since the germination of labeled asci with unlabeled glucose yields labeled glycerol (unpublished results).

The peak intensities of glucose and trehalose are plotted as functions of time in Fig. 3a. Note that trehalose intensities are increased by a factor of 10 for comparison. The initial intensi-

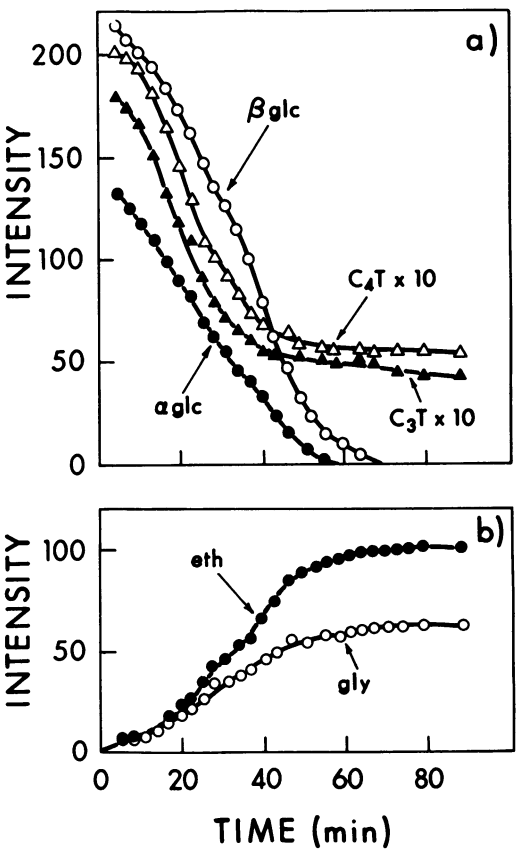

FIG. 3. Time courses of the germination of a 20% suspension of [^{13}C]trehalose-labeled asci with 75 mM [1-^{13}C]glucose in 50 mM Tris–5 mM potassium phosphate (pH 7.4) showing (a) glucose and trehalose consumption and (b) ethanol and glycerol production at 25°C.

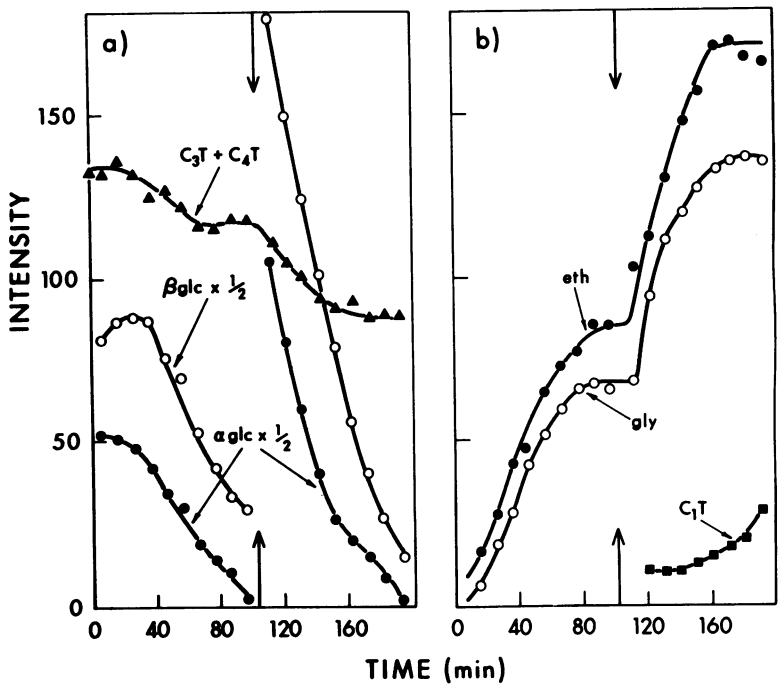

FIG. 4. Time course at 25°C during germination at a 4% suspension of ^{13}C-labeled asci showing (a) glucose and trehalose consumption and (b) the production of ethanol, glycerol, and trehalose, based on ^{13}C NMR spectra. ^{13}C NMR spectra were accumulated for 10 min; pulse intervals, 2.0 s. Initial [1-^{13}C]glucose feeding was at a concentration of 4.0 mM. Arrows indicate refeeding with 10 mM [1-^{13}C]glucose.

ties corresponded to a glucose concentration of 75 mM and an effective trehalose concentration in solution of 8 mM in the suspension or an intracellular concentration of 100 mM. These plots show that when glucose was first added both the glucose and the trehalose were rapidly consumed with similar kinetics. After approximately 50 min, when the glucose level had fallen to 10% of its original value, the trehalose, which had fallen to 25% of its initial value, leveled off and remained constant. In this experiment, therefore, the ratio of trehalose to glucose catabolized was 0.09. Trehalose, the endogenous carbohydrate, although decreasing substantially upon germination with glucose, was not preferentially catabolized, nor did it serve as the primary energy supply for the germination of the dormant spores. Figure 3b shows how labeled glycerol and ethanol followed carbohydrate consumption.

The relationship between trehalose mobilization and exogenous glucose level can be seen in Figure 4. The time course at 25°C of glucose and trehalose consumption of a low-density ascus suspension is plotted in Fig. 4a, and that of ethanol and glycerol production is plotted in Fig. 4b. Here [1-^{13}C]glucose was added at a low concentration of 4.0 mM. Whereas the glucose was essentially consumed entirely, the trehalose peak intensities decreased by only 12% and thereafter leveled off as the glucose concentration reached 2.4 mM. After 105 min, the asci were again fed [1-^{13}C]glucose at a concentration of 10 mM. Again trehalose consumption started, but when the glucose concentration reached ~2.4 mM trehalose consumption halted as previously. It appears that trehalose is only consumed when there is a sufficiently high level of glucose present.

The NMR experiments not only determine both trehalose and glucose consumption, but also monitor newly synthesized trehalose. Figure 5 shows a region of the ^{13}C NMR spectrum expanded about the C_1 glucose anomeric peaks for the low-density ascus suspension plotted in Fig. 4. This spectrum represents accumulations between 160 and 200 min after the initial glucose feeding. The peak centered at 94.0 ppm corresponds to newly formed [1-^{13}C]trehalose, and the time course of its formation is also plotted in Fig. 4b. The residual β-C-1 and α-C-1 peaks of glucose are seen at lower and higher fields (96.7 and 92.9 ppm) than the resolved C_1 trehalose peak. Because of the labeling site it is apparent that this peak corresponds to trehalose derived from [1-^{13}C]glucose and therefore was newly

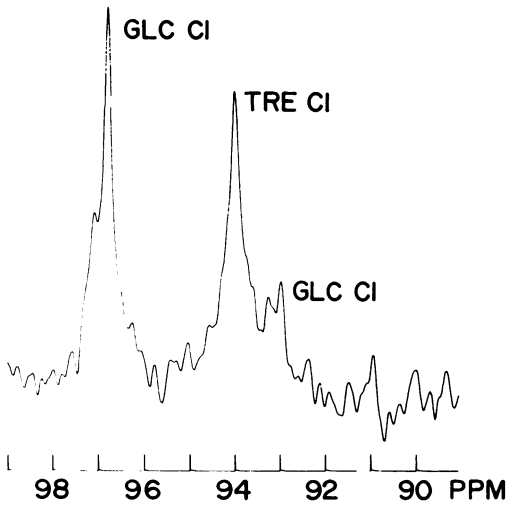

FIG. 5. Expanded spectrum for the germinated asci suspension of Fig. 4, showing the resolved peaks corresponding to the β and α C-1 positions of glucose and newly formed ^{13}C-labeled trehalose. This spectrum represents accumulations between 160 and 200 min after the initial glucose feeding. Pulse intervals, 2.0 s.

synthesized. It appears that if during germination the glucose level is lowered, thereby halting trehalose mobilization, trehalose may again be stored. It is noteworthy that in this experiment trehalose was only formed after the second glucose feeding, not at the equivalent time after the first feeding. Trehalose formation has also been observed shortly after glucose exhaustion in low-density experiments where the initial glucose concentration was 50 mM and after the trehalose had been substantially catabolized.

Figure 6 shows the NMR spectrum of a perchloric acid extract of labeled asci which had been incubated with [1-^{13}C]glucose for 15 min anaerobically. The extract spectra confirm the trehalose peak assignments made in the spectra of intact asci. The two triplet patterns for the C-3 and C-4 positions of singly and doubly labeled trehalose are even more clearly resolved. In addition, products of the [1-^{13}C]glucose catabolism can be seen more clearly here than in the spectra of intact asci. Glutamate labeling in the C-2, C-3, and C-4 positions was evident, as well as alanine, succinate, and aspartate. Glycolytic intermediates were not observed, possibly in part due to the high concentrations of glucose whose naturally abundant ^{13}C peaks would obscure peaks of the low-concentration intermediates.

Since our experiments demonstrate that the endogenous trehalose is not a primary carbohydrate source for germination with glucose, we have examined other conditions under which trehalose may be mobilized. Using ^{13}C NMR and classical extraction and colorimetric assays (8), we have observed that trehalose levels decrease in spores when challenged with 2-deoxyglucose. A maximum decrease of 19% in trehalose levels is observed after incubation with up to 0.5 M 2-deoxyglucose for 18 h. Parallel ^{31}P NMR experiments indicate the formation of 2-deoxyglucose 6-phosphate during this period. By quantitating trehalose loss with ^{13}C NMR and [^{3}H]2-deoxyglucose incorporation in the asci (see above) in several trials, we found that 3.8 ± 0.4 molecules of 2-deoxyglucose are incorporated for each trehalose molecule consumed (Table 1). Since each trehalose molecule consumed through glycolysis should create four equivalents of ATP, this result corresponds to the expected stoichiometric ratio of trehalose usage to 2-deoxyglucose incorporation. Hence the experiments show that trehalose is not the dominant energy supply for spore germination in the presence of glucose, but trehalose can act as a carbohydrate source for ATP formation in the dormant yeast spores upon challenge with 2-deoxyglucose.

DISCUSSION

By incorporating a ^{13}C label into the endogenous trehalose pool, we have been able to monitor the mobilization of trehalose in yeast asci during germination and dormancy. The results presented show that 80% of the trehalose is catabolized during the initial stages of germination with glucose. As has been seen previously (19), some trehalose does remain and may represent a different trehalose pool.

The possible roles for the high levels of trehalose found in fungal spores can be examined with respect to our results. Using [1-^{13}C]glucose, we have followed simultaneously the catabolism of the exogenous glucose and endogenous trehalose during the initial germination period. Our results show that trehalose does not

TABLE 1. Summary of ascus incubations with 2-deoxyglucose

Trial	Trehalose consumed (mmol)	2-Deoxyglucose incorporated (mmol)	mol of 2-deoxyglucose incorporated/mol of trehalose consumed
1	1.06	4.0	3.8
2	1.02	4.6	4.5
3	1.08	4.3	4.0
4	0.90	2.6	2.9
Avg			3.8 ± 0.4

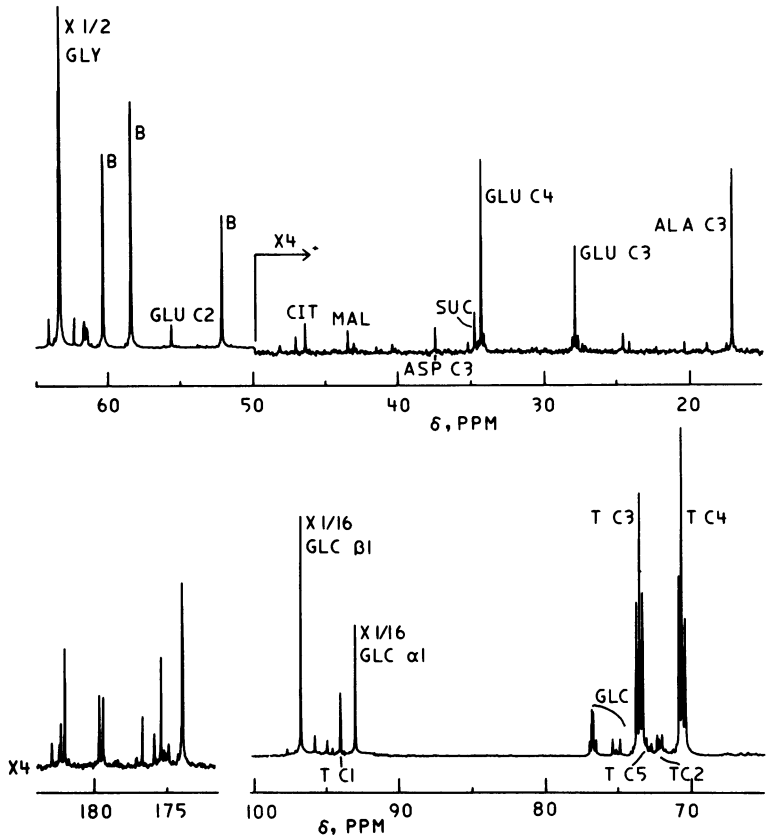

FIG. 6. Perchloric acid extract of ^{13}C-labeled asci (20% suspension) in 50 mM EDTA–10% D_2O at 25°C after 15 min of incubation with 65 mM [1-^{13}C]glucose. The spectrum represents 20,000 scans. Pulse intervals, 2.0 s; line broadening, 1 Hz. Abbreviations: GLC, glucose; T, trehalose; GLY, glycerol; GLU, glutumate; CIT, citrate; MAL, malate; ASP, aspartate; SUC, succinate; ALA, alanine; B, buffer peaks.

provide the primary energy supply during germination. Indeed, its usage accounts for only 9% of the carbohydrate consumed during germination, and the primary source of energy is the exogenous glucose. If trehalose is not needed as an energy source for germination, perhaps instead it is required during dormancy. Two sets of preliminary experiments show that trehalose can be catabolized during dormancy. First, during wet incubation periods of up to 2 months at room temperature, trehalose in the yeast asci decreased to ~25% of its initial values (unpublished results in our laboratory). Second, in the present study we have shown that in spores trehalose can be quantitatively catabolized to yield ATP when challenged with 2-deoxyglucose. Each trehalose molecule consumed results in the phosphorylation of four molecules of 2-deoxyglucose, presumably by synthesizing four ATP molecules. Thus trehalose is capable of mobilization as an energy source upon challenge with deoxyglucose and plausibly was being used for this purpose during dormancy. It is noteworthy that ATP levels in dormant asci are too low to be detected by ^{31}P NMR, i.e., appreciably below 1 mM, in contrast to the normal 5 mM concentration in energized yeast cells (12). However, their rate of production can be assayed by the rate at which 2-deoxyglucose is phosphorylated. Hence, these results support the hypothesis that the function of trehalose is to serve as an energy source during dormancy. As such it is consistent with the idea that trehalose is needed for the survival of the spore, not unlike the wet heat resistance found in other fungi (6). In this case, during germination, when no longer required, the trehalose is quickly catabolized.

The present results also illustrate some aspects of the control of trehalose catabolism. During germination trehalose mobilization appears to be regulated by the external glucose concentration. The K_m values for glucose in

catabolite-repressed cells, i.e., cells grown on glucose, are 8 and 5 mM for the α and β anomers of glucose, respectively (4). The present results show that when glucose levels drop below several millimolar, trehalose consumption stops. Although the critical glucose level cannot be equated with the unknown K_m of germinating spores, it is clear that the critically controlling level of glucose is in the vicinity of the glucose K_m in vegetative cells. A second experiment showed the same correlation; newly synthesized trehalose from the [1-^{13}C]glucose appeared when glucose was almost exhausted 120 min after germination started (Fig. 4b). Here the results show that low glucose levels do not cause trehalose to be hydrolyzed and in fact allow it to be synthesized. A third apparently consistent result, linking glucose levels to trehalase activity, is observed during sporulation, when an absence of glucose leads to trehalose synthesis (from acetate). These results, although showing a consistent relationship between glucose levels and trehalose formation, lead to difficulties if we try to postulate a role for cyclic AMP (cAMP) in the glucose control of trehalase activity. It has been suggested by Van der Plaat and Van Solingen (24, 25), that cAMP activates trehalase in yeast, in a fashion quite analogous to its stimulation of phosphorylase. If this were operative in our experiments then we would have to postulate that in all three states mentioned the cAMP levels followed the glucose levels, i.e., high glucose gave high cAMP. This, however, is not consistent with the widespread view that high glucose levels suppress cAMP levels (1). If we follow this more usual assumption, which is the basis of glucose repression, then we need a mechanism to explain the present results. A possible series of mechanism is that low glucose increases cAMP which then increases the concentration or activity of the trehalose synthetase-trehalase complex, as has been suggested by Panek and Mattoon (16). However, in this model cAMP would not increase the rate of degradation of trehalose by trehalase. This model would explain our observed cases of trehalose formation, i.e., during sporulation and after glucose exhaustion (Fig. 4b) by the presence of high cAMP levels, brought about by glucose deprivation.

To explain the disappearance of trehalose upon glucose addition to spores it is not sufficient to say that trehalose synthesis is inhibited, since it presumably has been during dormancy. Here we think it likely, as previously suggested, that during dormancy compartmentation of trehalose and trehalase has prevented degradation, and that this compartmentation is lost by the morphological changes occurring during germination. This is based upon evidence (21, 23) that in yeast and other fungi, under non-proliferating conditions, trehalase and trehalose are in separate compartments of the cell. Although our results suggest several possible controlling factors for trehalose catabolism, the relations of ATP, cyclic AMP, and compartmentation, all of which are possible controlling factors of trehalose metabolism, remain to be determined (7, 17).

ACKNOWLEDGMENT

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